CLAIMS

What is claimed is:

1	1. A method for determining the effect of a test agent on a tissue engineered				
2	cartilage matrix, comprising:				
3	(A) culturing an engineered cartilage tissue comprising the steps of:				
4	(i) culturing isolated chondrogenic cells for an amount of time effective				
5	for allowing formation of a chondrogenic cell-associated matrix; and				
4 7 8 9 9 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4	(ii) culturing the chondrogenic cells with the cell-associated matrix on a				
	semipermeable membrane in the presence of a growth factor for a time effective for allowing				
	formation of the engineered cartilage tissue;				
9	(B) contacting one or more test agents with one or more cells or tissues selected				
1 0	from the group consisting of (a) the isolated chondrogenic cells prior to (i), (b) the chondrogenic				
5 1	cells during (i), (c) the chondrogenic cells and cell-associated matrix prior to (ii), (d) the				
	chondrogenic cells and cell-associated matrix during (ii), and (e) the engineered cartilage tissue;				
14 143	and				
112 113 114	(C) measuring the effect the one or more test agents has on the contacted cells or				
15	tissue.				
1	The method of claim 1 wherein the chandro conjugated matrix				
1	2. The method of claim 1 wherein the chondrogenic cell-associated matrix				
2	comprises aggrecan, collagen types II, IX and XI, matrix proteins and hyaluronan.				
1	3. The method of claim 1 wherein the engineered cartilage tissue comprises				
2	collagen types II, IX and XI, hyaluronan and at least about 5 mg/cc ³ aggrecan,				
3	wherein the ratio of aggrecan to hyaluronan is about 10:1 to about 200:1, and the				

ratio of aggrecan to collagen is about 1:1 to about 10:1.

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1	4. The method of claim 1 wherein the isolated chondrogenic of	cells are from			
2	articular cartilage.				
1	5. The method of claim 1 wherein the isolated chondrogenic	cells are from			
2					
3	cartilage, arytenoid cartilage or cricoid cartilage.				
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1	6. The method of claim 1 wherein the isolated chondrogenic	cells are from			
2	2 fibrocartilage.				
1	7. The method of claim 6 wherein the fibrocartilage is ligame	ent, tendon,			
<u>1</u> 2	meniscus or intervertebral disc.				
I 1	8. The method of claim 1 wherein step (i) comprises culturing	g the			
	chondrogenic cells on an alginate medium.				
a.d ■ 1	9. The method of claim 1 wherein step (C) comprises measur	ing the amount			
5 2	of proteoglycan in the engineered cartilage tissue.				
	10. The method of claim 1 wherein step (C) is performed with	out the addition			
TU 2	2 of extrinsic radioactivity.				
1	11. The method of claim 10 wherein step (C) comprises enzym	natically			
2	degrading the engineered cartilage tissue.				
1	12. The method of claim 11 wherein step (C) further comprise	s staining the			
2	enzymatically degraded engineered cartilage tissue with a dye.				
1	13. The method of claim 1 wherein the engineered cartilage tis	ssue is removed			
2	from the seminermeable membrane prior to being contacted with the test agent.				

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1	14.	The method of claim 1 further comprising:		
2	(D) identifying one or more test agents that have desirable properties; and			
3	(E) producing the one or more test agents as a therapeutic drug.			
1	15. A	kit for determining the effect of a test agent on a tissue engineered cartilage		
2	matrix comprising instructions for carrying out the method of claim 1.			
1	16.	The kit of claim 15 further comprising one or more of:		
2		(i) one or more reagents;		
3		(ii) an enzyme capable of degrading the engineered cartilage tissue;		
4		(iii) a dye capable of labeling a component of the engineered cartilage		
<u>l</u> 5	tissue; and			
D 6		(iv) an antibody capable of labeling a component of the engineered		
	cartilage tissue.			
1 5	17.	A method for determining the effect of a test agent on a tissue engineered		
_	cartilage matrix, comprising:			
	(A)	culturing an engineered cartilage tissue comprising the steps of:		
្រុំ ស្នូ 4		(i) culturing isolated chondrogenic cells for an amount of time effective		
5	for allowing formation of a chondrogenic cell-associated matrix; and			
6		(ii) culturing the chondrogenic cells with the cell-associated matrix on a		
7	semipermeable mem	brane in the presence of a growth factor for a time effective for allowing		
8	formation of the engineered cartilage tissue;			
9	(B)	contacting one or more test agents with one or more cells or tissues		
10	selected from the group consisting of (a) the isolated chondrogenic cells prior to (i), (b) the			
11	chondrogenic cells during (i), (c) the chondrogenic cells and cell-associated matrix prior to (ii),			
12	(d) the chondrogenic cells and cell-associated matrix during (ii), and (e) the engineered cartilage			
13	tissue in the presence of a known modulator of cartilage tissue; and			
14	(C)	measuring the effect the one or more test agents has on the contacted cells		
15	or tissue.			

1		18.	The method of claim 17 wherein the chondrogenic cell-associated matrix			
2	comprises aggrecan, collagen types II, IX and XI, and hyaluronan.					
	-	_				
1		19.	The method of claim 17 wherein the isolated chondrogenic cells are from			
2	articular carti	lage.				
1		20.	The method of claim 17 wherein the isolated chondrogenic cells are from			
2	costal cartilag	costal cartilage, nasal cartilage, auricular cartilage, tracheal cartilage, epiglottic cartilage, thyro				
3	cartilage, aryt	enoid o	cartilage or cricoid cartilage.			
1		21.	The method of claim 17 wherein the isolated chondrogenic cells are from			
_{1.1} 2	fibrocartilage.					
ld In		22.	The method of claim 21 wherein the fibrocartilage is ligament, tendon,			
	meniscus or intervertebral disc.					
l-i						
		23.	The method of claim 17 wherein step (i) comprises culturing the			
5 2	chondrogenic cells on an alginate medium.					
ri Nj						
The second secon		24.	The method of claim 17 wherein the engineered cartilage tissue comprises			
<u>1</u> 2	collagen types II, IX and XI, hyaluronan and at least about 5 mg/cc ³ aggrecan,					
3		wherein the ratio of aggrecan to hyaluronan is about 10:1 to about 200:1, and the				
4	ratio of aggre	can to	collagen is about 1:1 to about 10:1.			
1		25.	The method of claim 17 wherein step (C) comprises measuring the amount			
2	of proteoglyca	an in th	ne engineered cartilage tissue.			
		26				
1		26.	The method of claim 17 wherein step (C) is performed without the			

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addition of extrinsic radioactivity.

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1	27.	The method of claim 26 wherein step (C) comprises enzymatically				
2	degrading the engir	rading the engineered cartilage tissue.				
1	28.	The method of claim 27 wherein step (C) further comprises staining the				
2	enzymatically degra	degraded engineered cartilage tissue with a dye.				
1 29. The method of claim 17 wher		The method of claim 17 wherein the modulator of the engineered cartilage				
2	tissue is a matrix st	imulating agent, cytokine or TNF-α.				
1	20	The method of claim 20 wherein the outoking is interloukin 1				
1	30.	The method of claim 29 wherein the cytokine is interleukin-1.				
<u>ļ</u> 1	31.	A kit for determining the effect of a test agent on an engineered cartilage				
	tissue comprising in	nstructions for carrying out the method of claim 17.				
, 1	32.	The kit of claim 31 further comprising one or more of:				
<u>⊧</u> ≟ 2		(i) one or more reagents;				
♣ 3		(ii) an enzyme capable of degrading the engineered cartilage tissue;				
1 4 1 4		(iii) a dye capable of labeling a component of the engineered cartilage				
TU 5	tissue; and					
5 M 6		(iv) an antibody capable of detecting a component of the engineered				
П 7	ivcartilage tissue.					
1	33.	The method of claim 17 further comprising:				
2	` '	(D) identifying one or more test agents that have desirable properties; and				
3	(E) I	producing the one or more test agents as a therapeutic drug.				
1	34.	The method of claim 17 further comprising removing the engineered				
2	cartilage tissue from the semipermeable membrane prior to contacting the engineered cartilage					
3	tissue with the test agent					

4 (3)

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1 35. The method of claim 17 wherein steps (A) and (B) occur in the same well

2 of a multiwell plate.